

AMENDMENTS TO THE SPECIFICATION

The following stated paragraphs replace all prior versions of these paragraphs in the application.

[0031] FIG. 8 shows the assay for PP2A phosphatase activity using Lissamine rhodamine labeled peptide substrate LRRApSLG SEQ ID NO: 1 at one or two hours of enzymatic reaction in a 96 well plate format. The data represent the means of triplicates and error bars show the standard deviation. The observed fluorescent signal increases with increasing amounts of enzymatic activity. The observed fluorescent signal is less quenched at each enzyme concentration with two hours of enzymatic reaction as compared to one hour of enzymatic reaction time, confirming the time-dependency of the enzymatic reaction.

[0035] FIGS. 12 and 13 shows the assay for proteolytic enzymatic activity of TPCK-treated trypsin using the Lissamine labeled peptide substrates LRRApSLG SEQ ID NO: 1 or AGLARAGLALARLALALRRApSL SEQ ID NO: 2, respectively. The observed fluorescence increases with increasing enzymatic activity, indicating proteolytic cleavage at a peptide bond N-terminal to the phosphoserine residue.

[0067] PKC (protein kinase C) assays were carried out in a reaction mixture consisting of 20 mM HEPES, 5 mM CaCl.sub.2, 5 mM MgCl.sub.2, 1 mM ATP (disodium salt), 1 mM DTT (dithiothreitol), 0.2 mg/ml phosphatidyl-L-serine, pH 7.4. The enzyme preparation (purified PKC containing alpha, beta, and gamma isoforms, Pierce Biotechnology, Rockford, Ill.) was diluted in 20 mM HEPES, 0.05% Triton X-100, pH 7.4, immediately before use in the assay. The enzyme substrates used were either Myelin Basic Protein Peptide 4-14 (EKPPSQRSKYL SEQ ID NO: 3) or Glycogen Synthase Peptide (PLSRTLSTVAKK SEQ ID NO: 4) or Pseudosubstrate Peptide (RFARKGSLRQKNV SEQ ID NO: 5) labeled on the N-terminal amine with Lissamine Rhodamine.

[0068] PKA (protein kinase A) assays were carried out in a reaction mixture consisting of 20 mM HEPES, 0.1 mM cAMP, 5 mM MgCl.sub.2, 1 mM ATP (disodium salt), 1 mM DTT, pH 7.4. The enzyme preparation (PKA catalytic unit, Promega, Madison, Wis.) was diluted in 20 mM HEPES, 0.05% Triton X-100, pH 7.4, immediately before use in the assay. The enzyme substrate was Kemptide having a sequence of LRRASLG SEQ ID NO: 6 and labeled on the N-terminal amine Lissamine Rhodamine.

[0069] Tyrosine kinase (Src p60c-src, partially purified, Upstate Biotechnology, Waltham, Mass.) assays were carried out in a reaction mixture consisting of 20 mM HEPES, 5 mM MgCl.sub.2, 1 mM ATP (disodium salt), 1 mM DTT, pH 7.4. The enzyme substrate Tyrosine Kinase Peptide having a sequence of KVEKIGEGTYGVYK SEQ ID NO: 7 and labeled on the N-terminal alpha amine with Lissamine Rhodamine.

[0075] PTP-Beta and PTP-1B (commercially available protein tyrosine phosphatases) assays were carried out in a reaction mixture consisting of 20 mM HEPES, 1.5 mM DTT, and 0,5 mM EDTA, pH 7.4. The enzyme substrate used for both enzymes was Lissamine Rhodamine labeled on the N-terminal amine of the following phosphotyrosine peptide substrate (~~KVEKIGEGT~~pYGVVYK SEQ ID NO: 8).

[0076] A PP2A (protein serine/threonine phosphatase) assay was carried out in a reaction mixture consisting of 20 mM HEPES, 1.5 mM DTT, pH 7.4. The enzyme substrate was Lissamine Rhodamine labeled on the N-terminal amine of phospho Kemptide having a sequence of ~~LRRApSLG~~ SEQ ID NO: 1.

[0081] The protease, Trypsin (TPCK treated), from Pierce Biotechnology, Inc., which cleaves peptide substrates C-terminal of arginine and lysine residues, was assayed (BAEE units/mg protein) in a reaction mixture consisting of 25 mM TRIS, 150 mM NaCl, pH 7.2. The enzyme substrates used were Lissamine Rhodamine labeled on the N-terminal amine of either phosphoKemptide having a sequence of ~~LRRApSLG~~ SEQ. ID. NO. 1 or a longer derivative thereof having the sequence ~~AGLARAGLALARLALALRRApSL~~ SEQ ID NO: 2.